Journal of Environment & Biotechnology Research

VINANIE PUBLISHERS
www.vinanie.com/jebr

Bioaccumulation of heavy metals in steel processing industrial effluents using *Bacillus safensis* LAU 13

S.O. Ojoawo,[1] A. Lateef,[2,*] F.A.Oyeniran,[1] O.T.Kupoluyi,[1] O.S.Opatola,[1] J.O.Daramola[1]

[1] Department of Civil Engineering, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria
[2] Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Industrial effluent often contains heavy metals in toxic concentrations which require remediation for environmental safety purposes. Bioremediation can be used as an effective means of reducing the heavy metal concentration in wastewater. This paper focuses on remediation by biosorption as an effective technique for reduction in the concentration of Cu, Mn, Co, Fe, Zn, Hg, Se, Cd, Pb and Cr present in wastewater samples of a foundry facility in Ogbomoso and a steel/ wire manufacturing industry in Ibadan, Southwest, Nigeria. Initial concentrations of heavy metals in the wastewater samples after digestion, were determined using atomic absorption spectrometer (AAS). The glucose enriched wastewater samples were then subjected to bio-treatment using *Bacillus safensis* LAU 13 (GenBank accession number KJ461434). The residual pellets after treatment were subjected to scanning electron microscopy (SEM). At the end of 48 h of growth, biosorption by *B. safensis* resulted in 100 % Co remediation and 82.2 - 98.97 % of Mn removal. In steel rolling mill effluent, *B. safensis* exhibited 97.68 % Se remediation, while removal ranging between 44 and 55 % were obtained for Cu, Fe and Zn. SEM micrographs showed the presence of remediated metals in form of aggregates in the bacterial biomass. This study established the biosorption technique with *B. safensis* LAU 13 as biosorbent as a viable method in the treatment of industrial wastewater in terms of material availability, cost-effectiveness and absence of precipitates and slurry.

KEYWORDS
Bacillus safensis; biosorption; heavy metals; industrial wastewater

1. INTRODUCTION

Water is vital to the survival of all living things as it makes up 50-97 % of the weight of plants and animals and about 70 % of human body. Presence of heavy metals in various quantities in industrial waste water has posed several challenges to the disposal, recycling, re-usage of the waste water, and also a major threat to the environment (Fola et al., 2016). Toxic heavy metal contamination in wastewaters is a worldwide problem (Kanchana et al., 2014). The persistent presence of these metals in water even in small quantities may become toxic through natural processes such as bio-magnification.

Industrial wastewaters represent significant sources in the pollution of water, where obnoxious and xenobiotic materials contaminate surface and ground water. Amongst such unwanted materials, heavy metals are major important polluting agents, such as As, Fe, Cu, Hg, Cd, Cr, Ni, Hg, Mn and Zn which are toxic to aquatic organisms as well as human beings (Eletta, 2012). Aside, pollution by heavy metals is also a threat to agriculture, other food sources for human population as well as poor vegetation development and reduction in resistance of plant to pests, thereby have great impact on the quality of food, ground water, soil microorganisms and plant growth (Ene et al., 2010; El-Bouraie et al., 2010; Bhagure and Mirgane, 2011). Removal of these heavy metals from wastewater is therefore very essential and of great importance for the

Corresponding author: **A. Lateef**
Tel: +234-803-740-0520
E. mail: alateef@lautech.edu.ng; agbaje72@yahoo.com

Received: 02-10-2016
Revised: 05-11-2016
Accepted: 29-11-2016
Available online: 01-01-2017

protection of environmental health (El-Sheltawy et al., 2016), hence should be given much attention. Removal or recovery of metals of technological importance or economic value from industrial wastewaters may be achieved using suitable treatment methods. Mostly, heavy metals are soluble in water, and consequently cannot be removed by ordinary physical means of separation (Oluyemi et al., 2010; Rajaganapathy et al., 2011).

Bioremediation is an optional method for the removal and detoxification of various pollutants through the use of biological entities (Adeogun et al., 2016; Rangabhashiyam and Balasubramanian, 2016). It is characterized by relatively low cost, low technology, and wide acceptance among the public. However, the major drawbacks of bioremediation methods include its ineffectiveness against range of contaminants, slower rate of remediation, and the desired residual levels of contaminants may not be achieved. Among several biological means of wastewater treatment, biosorption using microbial cells is a viable process.

Bacillus safensis is a spore-forming Gram-positive, mesophilic and aerobic bacterium that exhibits chemo-heterotrophic nutrition. The rod shaped bacterium is capable of motility, and can tolerate high levels of salinity, heavy metals, as well as radiations (Satomi et al., 2006; Raja and Omine, 2012; Kothari et al., 2013; Lateef et al., 2015a). *B. safensis* thrives in wide range of environments, including those considered unsuitable for the survival of other organisms. Strains of the bacterium have been isolated from diverse sources such as polluted soils, effluents, desert, excreta, plant parts, spacecraft and related environments. The first isolation of *B. safensis* by Satomi et al. (2006) was from spacecraft-assembly facility (SAF) at the Jet Propulsion Laboratory, USA. Numerous reports have indicated that *B. safensis* can grow under wide range of temperature (10-50 ^{o}C) and pH (4.0-9.0). However optimum growth is achieved at temperature of 37 ^{o}C and pH of 7.0 (Satomi et al., 2006; Singh et al., 2013; Kothari et al., 2013; Roohi et al., 2014).

Physiologically, *B. safensis* can serve as tremendous sources of enzymes that are of importance to industries. There were reports based on the production of enzymes such as amylase, lipase, cellulase, protease, β-galactosidase, endoinulinase and keratinase by some isolates of *B. safensis* (Kothari et al., 2013; Singh et al., 2013; Reza et al., 2014; Lateef et al., 2015b). We have recently reported the production of keratinase by a new isolate of *B. safensis* LAU 13 in our laboratory (Lateef et al., 2015b; Adelere and Lateef, 2016). The isolate was found to be capable of degrading whole feather, and its keratinase used for dehairing of goat skin, removal of blood stain and biosynthesis of silver, gold and silver-gold alloy nanoparticles (Lateef et al., 2015a-d; Ojo et al., 2016; Lateef et al., 2016). There were also reports about the use of *B. safensis* to produce some secondary metabolites; including arachidonic acid, carotenoids and biosurfactants (Khaneja et al., 2010; Porob et al., 2013; Goncharova et al., 2013) for diverse applications, which was documented in the first review on the biology and biotechnological applications of *B. safensis* (Lateef et al., 2015a).

The present investigation is a further attempt at expanding the frontiers of application of *B. safensis* LAU 13, in the biosorption of heavy metals from the wastewater of steel-processing facilities. To the best of our knowledge, this is the first report of the use of *B. safensis* in the biosorption of heavy metals from the wastewater of steel-processing plants.

2. MATERIALS AND METHODS

2.1. Wastewater

The preliminary experimental study was conducted on the wastewater samples collected from the foundry facility in Ogbomoso and a steel/wire manufacturing industry in Ibadan, Southwest, Nigeria. The samples were collected using clean 2 L plastic containers, tightly corked and taken directly to the Laboratory for analyses. Digestion was carried out on the wastewater sample with the aim of breaking down the complexity of the sample before the AAS analysis. About 10 mL of the wastewater was dispensed into a beaker and 10 mL of concentrated (70 wt%, 15.7 N) HNO_3 was added to it to stimulate a rapid reaction (Sherrod et al., 1999). The wastewater-nitric acid mix was placed inside the fume cupboard and heated with heating mantle for 30 min at 100 ˚C. Thereafter, the sample was removed from the fume cupboard. Distilled water was then added to the sample making it up to 100 mL before it was filtered through Whatman filter paper No. 1, and the filtrate was poured inside a container for the atomic absorption spectrometry (AAS) analysis to determine the initial and final concentrations of heavy metals present in the wastewater samples and in the control sample. The instrument used was flame AAS with Model number PG990. The equipment was adjusted each time to the wavelength of metal to be analyzed while its mono-chromator measures the quantities of the absorbed metals. The flame used in the analysis was air-acetylene, producing flame of around 2300 ˚C. The FAAS technique made use of the fact that neutral or ground state atoms of an element can absorb electromagnetic radiation over a series of very narrow, sharply defined wavelengths (Ojoawo and Udayakumar, 2015).

2.2. Bacterium

A strain of *Bacillus safensis* LAU 13 (GenBank accession number KJ461434) that was isolated from poultry feather waste (Lateef et al., 2015b) was used for bioremediation of steel industry wastewater in the present work. It was maintained on yeast extract agar and stored at 4 °C.

2.3. Biosorption studies

The initial pH of the wastewater was determined using digital Systronics pH meter, System 361 model and then adjusted to 7.5 with 1 M NaOH. The wastewater samples were supplemented with 1.0 g/L of glucose to enhance the growth of the bacterium (Ojoawo et al., 2016). The supplemented waste water samples were then dispensed as 10 mL into test tubes and autoclaved at 121 °C for 15 min. The sterilized supplemented wastewater samples were then inoculated with 250 µL of 18 h broth culture of *B. safensis* LAU 13 containing approximately 1 × 10^6 cfu/mL. The cultures were incubated at 37 °C on rotary shaker at 100 rpm for 24-120 h. At 24 h interval, samples were withdrawn for the determination of optical density at 600 nm as a measure of growth. Thereafter, samples were centrifuged at 4000 rpm for 15 min, and the supernatants were subjected to AAS analysis to determine the residual concentration of heavy metals. Furthermore, samples for scanning electron microscopy (SEM), taken from the residual pellets after the remediation process, were prepared by carbon taping. They were stuck on the carbon tape plate and subjected to detail-obscuring conductive gold coating (Ojoawo et al., 2016) and subsequently exposed to SEM analysis.

3. RESULTS AND DISCUSSION

3.1. Wastewaters from foundry and steel rolling mill

The results of the concentrations of heavy metals in the two industrial wastewaters are as presented in Table 1. The concentrations ranged from 0.097mg/L for Co^{2+} to 12.361 mg/L for Fe^{2+} in the foundry wastewater, while range of 0.00 mg/L for Cr^{3+} to 174.410 mg/L for Fe^{2+} were obtained for the steel rolling effluent. The measured concentrations of metals in the effluent is justified by the fact that foundry is recognized as one of the most complex technologies in manufacturing industry that includes preparation and processing of alloys, metals and non-metallic materials while being cast into desired forms. The steel rolling industrial activities as well expectedly produce worn metals contained in its residual wastewater, especially as a result of the water cooling process (Adelekan and Abegunde, 2011). At present, the two sources of wastewater do not have standard treatment facilities as a result of which the effluents are channeled into adjoining seasonal streams. Comparison of the initial concentrations of heavy metals in this study with the EPA Standard (Ojoawo and Udayakumar, 2015) indicates a very high level of pollution. For humans, Singh et al. (2011) presented permissible levels for As, Cd, Pb, Mn, Hg and Zn as 0.02, 0.06, 0.1, 0.26, 0.01 and 15 mg/L respectively. It was observed from this

Table 1. Initial heavy metal concentrations (mg/L) of the industrial wastewaters.

Metal	Symbol	Foundry	SD	Steel rolling	SD
Copper	Cu^{2+}	0.353	0.0013	0.246	0.0010
Manganese	Mn^{2+}	1.070	0.0013	1.828	0.0006
Cobalt	Co^{2+}	0.097	0.0008	0.030	0.0008
Iron	Fe^{2+}	12.361	0.0007	174.410	0.0009
Zinc	Zn^{2+}	1.045	0.0008	12.214	0.0007
Mercury	Hg^{2+}	0.158	0.0008	0.169	0.0006
Selenium	Se^{4+}	4.086	0.0012	0.604	0.0013
Cadmium	Cd^{2+}	0.440	0.0007	0.276	0.0010
Lead	Pb^{2+}	1.957	0.0007	1.071	0.0013
Chromium	Cr^{3+}	1.261	0.0009	0.000	0.0011

study that only the concentration of Zn falls within the permissible limit. Indiscriminate discharges of such improperly treated effluents constitute pollution of the ecosystem and may negatively affect human health (Wufem et al., 2009; Obodai et al., 2011).

3.2. Biosorption of heavy metals by B. safensis LAU 13

The results of the biosorption of heavy metals by *B. safensis* LAU 13 are presented in Figure 1. Among the metals, removal of 26.6 and 100 % were achieved for Hg^{2+}, and Co^{2+} respectively in the two effluents. However, higher percentage of removal of Cu^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, and Pb^{2+} were recorded in foundry effluents than the steel rolling wastewater. In general, more than 50 % reduction was achieved for at least six of the heavy metals in each of the wastewaters at the end of 48 h growth of *B. safensis*. The difference in the efficiency of biosorption of heavy metals in the two wastewaters may be attributed due to the different concentrations of metals initially present in the effluents, the occurrence of some other metals not analyzed in this study, and interactive toxic effects of the components on the microbial biomass.

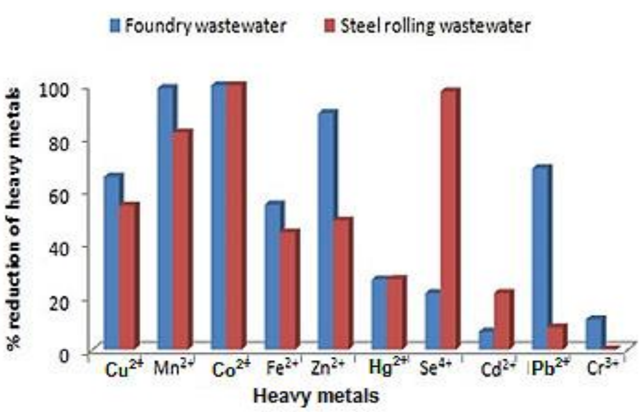

Figure 1. Attenuation of metals by biosorption using *B. safensis* LAU 13 in the selected effluents.

The use of bacteria for biosorption of heavy metals are widely reported in literature (Yilmaz and Ensari, 2005; Kinoshita et al., 2013; Özdemir et al., 2013; Oves et al., 2013; Ojoawo et al., 2016), including the use of some strains of *B. safensis* for the remediation of nickel (Motesharezadeh and Savaghebi-Firoozabadi, 2011). Also, there were reports regarding the isolation of metal and salt-tolerant strains of *B. safensis* (Raja and Omine, 2012; Kothari et al., 2013) as potential candidates for remediation of heavy metals. The high tolerance of the bacterium to salt, radiations and heavy metals (Satomi et al., 2006; Raja and Omine, 2012; Kothari et al., 2013), promotes the survival of the bacterium in such environments where other bacterial cannot grow or survive. Results obtained in this study showed that *B. safensis* can tolerate the presence of heavy metals, and also remediate same from industrial effluents. Several of our previous studies have shown the abilities of keratinase and cell-free extract of the bacterium to reduce silver, gold and silver-gold ions into their respective nanoparticles (Lateef et al., 2015c, d; Lateef et al., 2016; Ojo et al., 2016). It is well known that microbial cells can attenuate toxicity of metals by reducing them into less toxic metallic nanoparticles. The SEM micrographs of residual pellets (Figure 2) clearly reveals the irregularly shaped fine particles and porosity surface texture with small aggregates onto which biosorption by *B. safensis* might have occurred. There were inter-sparse deposits observed to be the remediated heavy metals from the wastewater samples. This is similar to findings of earlier research studies (Zhao et al., 2010; Shahjee et al., 2013; Yao et al., 2014).

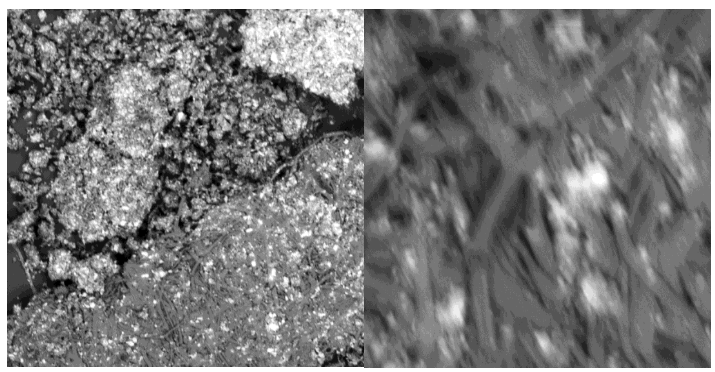

Figure 2. SEM micrograph of residual pellets after the bio-sorption process at X75 (left) and X500 (right).

4. CONCLUSIONS

This study has shown that *Bacillus safensis* LAU 13 exhibited effective capacity to reduce the toxic contents of foundry industrial wastewater and the steel rolling effluent to a bearable minimum level. The addition of glucose to the foundry industrial wastewater and the steel rolling effluent enhanced the bioremediation processes of the wastewater samples. As justified by the biosorption of the metals and the SEM micrographs, it can therefore be concluded that the bacterium could be a potential candidate for bioremediation of industrial effluents.

ACKNOWLEDGEMENTS

SOO and AL thank authority of LAUTECH, Ogbomoso, Nigeria for provision of some of the facilities used in this study. The Department of Materials Science and Engineering at Kwara State University, Malete, Nigeria is appreciated for the SEM analysis.

REFERENCES

Adelekan, B.A. and Abegunde, K.D. (2011) Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria. International Journal of Physical Sciences, 6, 1045-1058.

Adelere, I.A. and Lateef, A. (2016) Keratinases: emerging trends in production and applications as novel multifunctional biocatalysts. Kuwait Journal of Science, 43, 118-127.

Adeogun, A.I., Sunday, O. and Olateju, K.S. (2016) Removal of Mn(II) from aqueous solution by *Irvingia gabonensis* immobilized *Aspergillus* sp. TU-GM14 isothermal, kinetics and thermodynamic studies. Journal of Environment and Biotechnology Research, 3, 1-11.

Bhagure, G.R. and Mirgane, S.R. (2011) Heavy metal concentrations in groundwaters and soils of Thane region of Maharashtra, India. Environmental Monitoring and Assessment, 173, 643-652.

El-Bouraie, M.M., El-Barbary, A.A., Yehia, M.M. and Motawea, E.A. (2010) Heavy metal concentrations in surface river water and bed sediments at Nile Delta in Egypt. Suo, 61, 1-12.

Eletta, B.E. (2012) Investigation of the extent of heavy metal pollution in sediments and sludge using atomic absorption spectroscopy (AAS). Journal of Nuclear and Particle Physics, 2, 87-90.

El-Sheltawy, S.T., El-shafei, M.M., El-deen, S. and Rasha, A. (2016) Evaluation of copper adsorption from industrial wastewater using sewage sludge ash. Journal of Environment and Biotechnology Research, 4, 24-31.

Ene, A., Bosneaga, A. and Georgescu, L. (2010) Determination of heavy metals in soils using XRF technique. Romanian Journal of Physics, 55, 815-820.

Fola, A.T., Idowu, A.A. and Adetutu, A. (2016) Removal of Cu^{2+} from aqueous solution by adsorption onto quail eggshell: Kinetic and isothermal studies. Journal of Environment and Biotechnology Research, 5, 1-9.

Goncharova, A.V., Karpenyuk, T.A., Tsurkan, Y.S., Beisembaeva, R.U., Kalbaeva, A.M., Mukasheva, T.D. and Ignatova, L.V. (2013) Screening and identification of microorganisms-potential producers of arachidonic acid. Proceedings of World Academy of Science, Engineering and Technology, 79, 1348-1351.

Kanchana, S., Jeyanthi, J., Kathiravan, R. and Suganya, K. (2014) Biosorption of heavy metals using algae: a review. International Journal of Pharma Medicine and Biological Sciences, 3, 1-9.

Khaneja, R., Perez-Fons, L., Fakhry, S., Baccigalupi, L., Steiger, S., To, E., Sandmann, G., Dong, T.C., Ricca, E., Fraser, P.D. and Cutting, S.M. (2010) Carotenoids found in *Bacillus*. Journal of Applied Microbiology, 108, 1889-1902.

Kinoshita, H., Sohma, Y., Ohtake, F., Ishida, M., Kawai, Y., Kitazawa, H, Saito, T. and Kimura, K. (2013) Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. Research in Microbiology, 164, 701-709.

Kothari, V.V., Kothari, R.K., Kothari, C.R., Bhatt, V.D., Nathani, N.M., Koringa, P.G., Joshi, C.G. and Vyas, B.R.M. (2013) Genomic sequence of salt-tolerant Bacillus safensis strain VK, isolated from saline desert area of Gujarat, India. Genome A, 1, 671-713.

Lateef, A., Adelere, I.A. and Gueguim-Kana, E.B. (2015a) The biology and potential biotechnological applications of *Bacillus safensis*. Biologia, 70, 411-419.

Lateef, A., Adelere, I.A. and Gueguim-Kana, E.B. (2015b) *Bacillus safensis* LAU 13: a new source of keratinase and its multi-functional biocatalytic applications. Biotechnology and Biotechnological Equipment, 29, 54-63.

Lateef, A., Adelere, I.A., Gueguim-Kana, E.B., Asafa, T.B. and Beukes, L.S. (2015c) Green synthesis of silver nanoparticles using keratinase obtained from a strain of *Bacillus safensis* LAU 13. International Nano Letters, 5, 29-35.

Lateef, A., Ojo, S.A. and Oladejo, S.M. (2016) Anti-candida, anti-coagulant and thrombolytic activities of biosynthesized silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13. Process Biochemistry, 51, 1406-1412.

Lateef, A., Ojo, S.A., Akinwale, A.S., Azeez, L., Gueguim-Kana, E.B. and Beukes, L.S. (2015d) Biogenic synthesis of silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13: antimicrobial, free radical scavenging and larvicidal activities. Biologia, 70, 1295-1306.

Motesharezadeh, B. and Savaghebi-Firoozabadi, G.R. (2011) Study of the increase in phytoremediation efficiency in a nickel polluted soil by the usage of native bacteria: *Bacillus safensis* FO.036b and *Micrococcus roseus* M2. Caspian Journal of Environmental Sciences, 9, 133-143.

Obodai, E.A., Boamponsem, L.K., Adokoh, C.K., Essumang, D.K., Villawoe, B.O., Aheto, D.W. and Debrah, J.S. (2011) Concentration of heavy metals in two Ghanaian lagoons. Archive of Applied Science research, 3, 177-187.

Ojo, S.A., Lateef, A., Azeez, M.A., Oladejo, S.M., Akinwale, A.S., Asafa, T.B., Yekeen, T.A., Akinboro, A., Oladipo, I.C., Gueguim-Kana, E.B. and Beukes, L.S. (2016) Biomedical and catalytic applications of gold and silver-gold alloy nanoparticles biosynthesized using cell-free extract of *Bacillus safensis* LAU 13: antifungal, dye degradation, anti-coagulant and thrombolytic activities. IEEE Transactions on NanoBioscience, 15, 433-442.

Ojoawo, S.O. and Udayakumar, G. (2015) Physico-chemical characterization of dry-weather-flow wastewater and assessment of treatment plants in Nitte and environs, India. IISTE Civil and Environmental Research Journal, 7, 56-65.

Ojoawo, S.O., Rao, C.V. and Goveas, L.C. (2016) Bioremediation of Zn, Cu, Mg and Pb in fresh domestic sewage by *Brevibacterium* sp. International Journal of Environmental Research, 10, 139-148.

Oluyemi, E.A., Adekunie, A.S., Adenuga, A.A. and Makinde, W.O. (2010) Physicochemical properties and heavy metal content of water sources in Ife north local government area of Osun State, Nigeria. African Journal of Environmental Science and Technology, 4, 691-697.

Oves, M., Khan, M. S. and Zaidi, A. (2013) Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. Saudi Journal of Biological Sciences, 20, 121-129.

Özdemir, S., Kılınç, E., Poli, A. and Nicolaus, B. (2013) Biosorption of heavy metals (Cd^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+}) by thermophilic bacteria, *Geobacillus thermantarcticus* and *Anoxybacillus amylolyticus*: equilibrium and kinetic studies. Bioremediation Journal, 17, 86-96.

Porob, S., Nayak, S., Fernandes, A., Padmanabhan, P., Patil, B.A., Meena, R.M. and Ramaiah, N. (2013) PCR screening for the surfactin (sfp) gene in marine *Bacillus strains* and its molecular characterization from *Bacillus tequilensis* NIO11. Turkish Journal of Biology, 37, 212-221.

Raja, C.E. and Omine, K. (2012) Arsenic, boron and salt resistant *Bacillus safensis* MS11 isolated from Mongolia desert soil. African Journal of Biotechnology, 11, 2267-2275.

Rajaganapathy, V., Xavier, F., Sreekumar, D. and Mandal, P. K. (2011) Heavy metal contamination in soil, water and fodder and their presence in livestock and products: A review. Journal of Environmental Science and Technology, 4, 234-249.

Rangabhashiyam, S. and Balasubramanian, P. (2016) Lignocellulosic biosorbents for the removal of hexavalent chromium from aqueous solutions: A review. Journal of Environment and Biotechnology Research, 5, 39-46.

Reza, K.M., Ashrafalsadat, N., Reza, R.M., Taher, N. and Ali, N. (2014) Isolation and molecular identification of extracellular lipase-producing *Bacillus* species from soil. Annals of Biological Research, 5, 132-139.

Roohi, A., Ahmed, I., Khalid, N., Iqbal, M. and Jamil, M. (2014) Isolation and phylogenetic identification of halotolerant/ halophilic bacteria from the salt mines of Karak, Pakistan. International Journal of Agriculture and Biology, 16, 564-570.

Satomi, M., Myron, T., Duc, L. and Venkateswaran, K. (2006) *Bacillus safensis* sp. nov., isolated from spacecraft and assembly-facility surfaces. International Journal of Systematic and Evolutionary Microbiology, 56, 1735-1740.

Shahjee, P., Godboley, B.J. and Sudame, A.M. (2013) Removal of fluoride from aqueous solution by using low cost adsorbent. International Journal of Innovative Research in Science, Engineering and Technology, 2, 2721-2725.

Sherrod, L., Maxwell, III, Sheldon, T. N. (1999) Method of digesting spent ion exchange resins and recovering actinides there from using microwave radiation. Online patents report accessed on 28th November, 2015 at www.google.com/patents/US5945342

Singh, R., Gautam, N., Mishra, A. and Gupta, R. (2011) Heavy metals and living systems: An overview. Indian Journal of Pharmacology, 43, 246-253.

Singh, R.S., Singh, R.P. and Yadav, M. (2013) Molecular and biochemical characterization of a new endoinulinase producing bacterial strain of *Bacillus safensis* AS-08. Biologia, 68, 1028-2013.

Wufem, B.M., Ibrahim, A.Q., Gin, N.S., Shibdawa, M.A., Adamu, H.M. and Agya, P.J. (2009) Levels of heavy metals in Gubi dam water Bauchi, Nigeria. Global Journal of Environmental Sciences, 8, 29-37.

Yao, S., Liu, Z. and Shi, Z. (2014) Arsenic removal from aqueous solutions by adsorption onto iron oxide/activated carbon magnetic composite. Journal of Environmental Health Science and Engineering, 12, 58.

Yilmaz, E.I. and Ensari, N.Y. (2005) Cadmium biosorption by *Bacillus circulans* strain EB1. World Journal of Microbiology and Biotechnology, 21, 777-779.

Zhao, X., Wang, J., Wu, F., Wang, T., Cai, Y., Shi, Y. and Jiang, G. (2010) Removal of fluoride from aqueous media by Fe_3O_4@ $Al(OH)_3$ magnetic nanoparticles. Journal of Hazardous Materials, 173, 102-109.